ADIPOSE-DERIVED STEM CELLS AND LATTICES

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BACKGROUND OF THE INVENTION

In recent years, the identification of mesenchymal stem cells, chiefly obtained from bone marrow, has led to advances in tissue regrowth and differentiation. Such cells are pluripotent cells found in bone marrow and periosteum, and they are capable of differentiating into various mesenchymal or connective tissues. For example, such bone-marrow derived stem cells can be induced to develop into myocytes upon exposure to agents such as 5-azacytidine (Wakitani et al., *Muscle Nerve,* 18(12), 1417–26 (1995)). It has been suggested that such cells are useful for repair of tissues such as cartilage, fat, and bone (see, e.g., U.S. Pat. Nos. 5,908,784, 5,906,934, 5,827,740, 5,827,735), and that they also have applications through genetic modification (see, e.g., U.S. Pat. No. 5,591,625). While the identification of such cells has led to advances in tissue regrowth and differentiation, the use of such cells is hampered by several technical hurdles. One drawback to the use of such cells is that they are very rare (representing as few as 1/2,000,000 cells), making any process for obtaining and isolating them difficult and costly. Of course, bone marrow harvest is universally painful to the donor. Moreover, such cells are difficult to culture without inducing differentiation, unless specifically screened sera lots are used, adding further cost and labor to the use of such stem cells. Thus, there is a need for a more readily available source for pluripotent stem cells, particularly cells that can be cultured without the requirement for costly prescreening of culture materials.

Other advances in tissue engineering have shown that cells can be grown in specially-defined cultures to produce three-dimensional structures. Spacial definition typically is achieved by using various acellular lattices or matrices to support and guide cell growth and differentiation. While this technique is still in its infancy, experiments in animal models have demonstrated that it is possible to employ various acellular lattice materials to regenerate whole tissues (see, e.g., Probst et al. *BJU Int.,* 85(3), 362–7 (2000)). A suitable lattice material is secreted extracellular matrix material isolated from tumor cell lines (e.g, Engelbreth-Holm-Swarm tumor secreted matrix—"matrigell"). This material contains type IV collagen and growth factors, and provides an excellent substrate for cell growth (see, e.g., Vukicevic et al., *Exp. Cell Res,* 202(1), 1–8 (1992)). However, as this material also facilitates the malignant transformation of some cells (see, e.g., Fridman, et al., *Int. J. Cancer,* 51(5), 740–44 (1992)), it is not suitable for clinical application. While other artificial lattices have been developed, these can prove toxic either to cells or to patients when used in vivo. Accordingly, there remains a need for a lattice material suitable for use as a substrate in culturing and growing populations of cells.

BRIEF SUMMARY OF THE INVENTION

The present invention provides adipose-derived stem cells and lattices. In one aspect, the present invention provides a lipo-derived stem cell substantially free of adipocytes and red blood cells and clonal populations of connective tissue stem cells. The cells can be employed, alone or within biologically-compatible compositions, to generate differentiated tissues and structures, both in vivo and in vitro. Additionally, the cells can be expanded and cultered to produce hormones and to provide conditioned culture media for supporting the growth and expansion of other cell populations. In another aspect, the present invention provides a lipo-derived lattice substantially devoid of cells, which includes extracellular matrix material from adipose tissue. The lattice can be used as a substrate to facilitate the growth and differentiation of cells, whether in vivo or in vitro, into anlagen or even mature tissues or structures.

Considering how plentiful adipose tissue is, the inventive cells and lattice represent a ready source of pluripotent stem cells. Moreover, because the cells can be passaged in culture in an undifferentiated state under culture conditions not requiring prescreened lots of serum, the inventive cells can be maintained with considerably less expense than other types of stem cells. These and other advantages of the present invention, as well as additional inventive features, will be apparent from the accompanying drawings and in the following detailed descriptions.

DETAILED DESCRIPTION OF THE INVENTION

One aspect of the invention pertains to a lipo-derived stem cell. Preferably, the stem cell is substantially free of other cell types (e.g., adipocytes, red blood cells, other stromal cells, etc.) and extracellular matrix material; more preferably, the stem cell is completely free of such other cell types and matrix material. Preferably, the inventive cell is derived from the adipose tissue of a primate, and more preferably a higher primate (e.g, a baboon or ape). Typically, the inventive cell will be derived from human adipose tissue, using methods such as described herein.

While the inventive cell can be any type of stem cell, for use in tissue engineering, desirably the cell is of mesodermal origin. Typically such cells, when isolated, retain two or more mesodermal or mesenchymal developmental phenotypes (i.e., they are pluripotent). In particular, such cells generally have the capacity to develop into mesodermal tissues, such as mature adipose tissue, bone, various tissues of the heart (e.g., pericardium, epicardium, epimyocardium, myocardium, pericardium, valve tissue, etc.), dermal connective tissue, hemangial tissues (e.g., corpuscles, endocardium, vascular epithelium, etc.), muscle tissues (including skeletal muscles, cardiac muscles, smooth muscles, etc.), urogenital tissues (e.g., kidney, pronephros, meta- and meso-nephric ducts, metanephric diverticulum, ureters, renal pelvis, collecting tubules, epithelium of the female reproductive structures (particularly the oviducts, uterus, and vagina)), pleural and peritoneal tissues, viscera, mesodermal glandular tissues (e.g., adrenal cortex tissues), and stromal tissues (e.g., bone marrow). Of course, inasmuch as the cell can retain potential to develop into mature cells, it also can realize its developmental phenotypic potential by differentiating into an appropriate precursor cell (e.g., a preadipocyte, a premyocyte, a preosteocyte, etc.). Also, depending on the culture conditions, the cells can also exhibit developmental phenotypes such as embryonic, fetal, hematopoetic, neurogenic, or neuralgiagenic developmental phenotypes. In this sense, the inventive cell can have two or more developmental phenotypes such as adipogenic, chondrogenic, cardiogenic, dermatogenic, hematopoetic, hemangiogenic, myogenic, nephrogenic, neurogenic, neuralgiagenic, urogenitogenic, osteogenic, pericardiogenic,